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Carbohydrate Polymers 66 (2006) 546–550

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Short communication

Ionic liquids as solvents for biopolymers: Acylation of starch and zein protein ☆

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Received 20 December 2005; received in revised form 24 March 2006; accepted 1 April 2006

Abstract

Biopolymers such as starch and zein protein were found to be soluble at 80 °C in ionic liquids such as 1-butyl-3-methylimidazolium chloride (BMIMCl) and 1-butyl-3-methylimidazolium dicyanamide (BMIMdca) in concentrations up to 10% (w/w). Higher concentrations of biopolymers in these novel solvents resulted in solutions with too high viscosity to stir. Solutions of both starch and zein in BMIMCl were acylated with anhydrides in presence of pyridine to give acetyl starch and benzoyl zein with various degrees of substitution. Without pyridine the acylation reaction did not proceed. ^{1}H NMR and IR spectroscopies were used to determine the degree of substitution of starch. Viscosity studies indicated that the starch underwent slight reduction in molecular weight during the course of acylation. Starch was also soluble in other non-conventional solvents such as choline chloride/oxalic acid and choline chloride/$ZnCl_2$. However, zein was insoluble in these solvents.

Keywords: Ionic liquid; Starch; Zein; Acetylation; Low melting; Deep eutectic mixtures

1. Introduction

Most of the organic and polymerization reactions are carried out in organic solvents of which some eventually end up polluting environment by evaporation or leakage. Recently, there have been intense research efforts to replace conventional organic solvents with novel solvent systems which are recyclable and environmentally compatible and therefore reduce waste and hazard. Ionic liquids (Seddon, 1997; Welton, 1999) and other non-conventional solvent systems such as choline chloride/oxalic acids (Abbott, Boothby, Capper, Davies, & Rasheed, 2004) and $ZnCl_2$ have drawn attention to the synthetic organic and polymer chemists worldwide, since they offer a potentially clean method to carry out chemical reactions or processes. They are non-volatile, while liquids over a wide range of temperatures, and offer benign alternatives to traditional organic solvents. To our knowledge, there is no prior report of the use of ionic liquid (IL) as solvent (Swatloski, Spear, Holbrey, & Rogers, 2002) for chemical modifications of starch and zein. Our goal is to investigate the solubility and reactions of these two biopolymers in these non-conventional solvents.

Among the industrial materials, starch is one of the cheapest and most cost-effective. In addition to being a major food (Stephen, 1995) item, it is currently used industrially (Whistler & BeMiller, 1993) as coatings and sizing in paper, textiles and carpets, as binders and adhesives, as absorbants, and as encapsulants. Starch is often modified to improve its end-use properties, e.g., oxidation, hydrolysis, and substitution with ethers, esters or quaternary

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0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.04.005

ammonium groups. However, the major hurdle to modify starch is due to its lack of solubility. The only solvent it is sparingly soluble in is dimethyl sulfoxide, which itself reacts with nucleophiles.

Zein (Lawton, 2002) is a naturally occurring protein polymer obtained as a product of industrial corn processing. In the past, fibers (Sturken, 1944) out of zein were used for garments, hats and other commercial applications. However, zein's chemical inertness and globular structure make molding articles difficult. A method to prepare zein acetate (Veatch, 1941) was reported in order to increase the water resistance, strength, and flexibility of zein films, coating or other bodies, but this method is useful for making acetyl derivative only i.e., zein acetate. Another major problem in modifying zein is also its lack of solubility. It dissolves in ethyl alcohol/water (90:10, w/w) mixture. However, this solution can not be employed for its chemical modification, since alcohol/water readily reacts with electrophilic reagents before zein does. To circumvent this problem, we found that *N*,*N*-dimethylformamide could be used as an inert solvent (Biswas, Sessa, Lawton, Gordon, & Willett, 2005; Biswas, Sessa, Gordon, Lawton, & Willett, 2005) for zein modification.

In this work, we sought to find inert solvents for biopolymers, such as starch and zein, so that they can be reacted in homogenous inert reaction media. This would enable us to chemically modify the biopolymer structures to improve their properties. The solutions of biopolymers could be reacted with many reactants that would have been impossible otherwise without the availability of inert solvents.

2. Experimental

2.1. Chemicals

The starch samples were obtained from Corn Products and the zein samples were from Freeman Industries. The acetic anhydride, pyridine and 1-butyl-3-methylimidazolium chloride, choline chloride, oxalic acid and $ZnCl_2$ were obtained from Aldrich Chemical Company. Sodium deuteroxide and deuterium oxide were obtained from Cambridge Isotope Laboratories, Inc., Andover, MA. All chemicals were used without further purification.

2.2. Instrumentation

Pierce Reacti-Vial™ glass vials, 10 mL, equipped with magnetic stirrer were used as reactors and Pierce Reacti-Therm™ heating block was used as the conventional heat source. Samples for IR spectra were prepared from 1 mg of powder and acquired on a Nicolet Avator 370 midrange Fourier Transform Infrared spectrometer using a SenIR Duroscope diamond ATR. NMR samples were prepared from 10 mg of product and 0.7 mL of D_2O and heated at 80 °C with stirring for 5 h. The ^1H NMR spectra were recorded with a Brüker DRX 400 spectrometer operating at 400 MHz.

2.3. Acetylation of starch

In a 10 mL Pierce Reacti-Vial™ glass vial equipped with magnetic stirrer, starch was added to the 1-butyl-3-methylimidazolium chloride at a concentration of 10% (w/w). To this mixture acetic anhydride and pyridine were added and the vial placed in a 100 °C Pierce Reacti-Therm™ heating block for 15 to 120 min.. The reaction mixture was precipitated with absolute ethanol then centrifuged at 3400 rpm for 25 min. The precipitate was washed with 5 mL of water, stirred for 10 min. and then 25 mL of absolute ethanol was added to precipitate the acetylated starch. Again we centrifuged and collected the residue. It was dispersed in absolute ethanol and filtered through Whatman No. 5 filter paper. The acetylated starch collected as residue was dried in a 35 °C in vacuum oven for 48 h. NMR solutions of starch acetates were prepared from 10 mg of product, 20 mg of 40% sodium deuteroxide and 0.7 mL of D_2O and heated at 80 °C with stirring for 1 h.

2.4. Benzoylation of zein

In glass vials, 100 mg of zein, 80 mg of benzoic anhydride and 30 mg of pyridine were mixed with 1 g of butyl-3-methylimidazolium chloride. This vial was heated to 120 °C for 60 min. The product, along with the excess reactants precipitated as a solid by washing with DI water. It was filtered, washed with hot water and crushed in a blender. The pasty solid turned into a yellow powder, which was filtered and washed with dilute hydrochloric acid to remove pyridine. Finally, the solid was washed three times with hot ethyl acetate to remove any organic compounds such as benzoic acid or pyridine. The zein sample was dried for 24 h in a 50 °C vacuum oven. NMR solution was prepared by warming a mixture of 10 mg zein esters and 10 mg of 40% sodium deuteroxide in 1 mL of deuterium oxide. The ^1H NMR spectrum for benzoylated zein in Fig. 3 showed aromatic peaks in the region of 7.5–8.0 ppm representing aromatic protons of benzoate group.

2.5. Degree of substitution (DS)

The DS of acetyl starch was determined by ^1H NMR spectroscopy using the ratio of the 1/3 three methyl protons absorbance at 1.9 ppm divided by 1/7 of the total starch CH areas i.e., at 3.3–3.9 (6H) and 5.2 (1H) ppm. Similarly, propionate DS was estimated by 1/2 of the two protons methylene protons at 2.09 ppm divided by 1/7 of the total starch CH protons.

2.6. Intrinsic viscosity

Intrinsic viscosities of starch esters hydrolyzed in 1 M KOH were determined following the method of Mark and Mehltretter (1970).

3. Results and discussion

Limited solubility of biopolymers restricts the number and nature of reagents that could be used for their chemical modifications. Starch is only soluble in DMSO and zein in alcohol/water (90:10, w/w). Alcohol and water are nucleophilic and their reactive nature precludes a large number of reagents that could have been used to modify these biopolymers. We found that both biopolymers are soluble in IL, BMIMCl, (Fig. 1) at 80 °C, up to 15% by weight. However, the viscosity increased so high that it was difficult to stir the solution. Thus, 10% solutions were reacted with anhydrides to demonstrate the utility of IL as a solvent media for the chemical modification of biopolymers. One disadvantage with BMIMCl is its high melting point of 69 °C.

Fig. 2 shows the photograph of 10% solutions of starch in IL and water. After heating at 95 °C for 40 min they were cooled to room temperature. A 10% (w/w) zein solution in BMIMCl was reacted with benzoyl chloride and pyridine to give benzoate derivative (Fig. 3). Acetylation of starch with acetic anhydride/pyridine was performed at 100 °C for 30 min. Similarly, propionic anhydride gave propionic ester.

Table 1 summarizes the effects of the amounts of acetic anhydride and pyridine on the product DS, yields and reaction efficiencies. Reaction efficiency indicates the fraction (in percent) of the total acetic anhydride that actually the reacts to form starch acetate. The DS was determined by ^1H NMR. The ^1H NMR starch acetate with DS 2.6 is shown in Fig. 4. Depending upon the amount of acetic anhydride and pyridine used we were able to get up to DS of 2.6. The reaction did not proceed in the absence of pyridine unlike the reported (Wu et al., 2004) acylation reaction of cellulose in IL that occurred without base. Intrinsic viscosities for starch acetates shown in Table 1 were 91, 94, 89, 76 and 77 mL/g for samples 1–5, respectively. These values are lower than the intrinsic viscosity for unmodified starch (160 mL/g), indicating some reduction in molecular weights probably due to acidic hydrolysis.

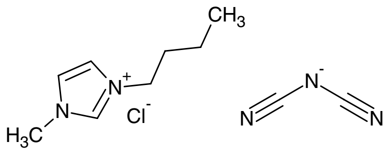

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Fig. 1. 1-butyl-3-methylimidazolium chloride (1) and dicyanamide anion (2).

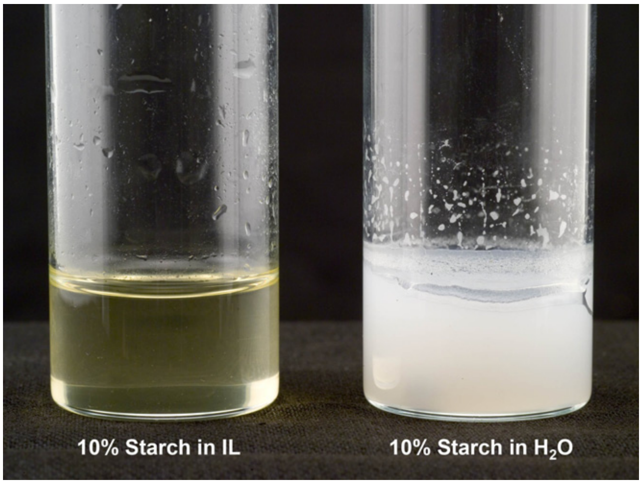

Fig. 2. Picture of 10% solutions of starch in IL and water.

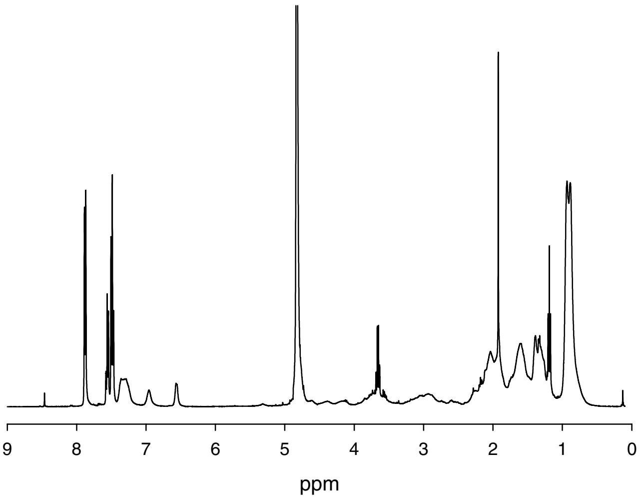

Fig. 3. Proton NMR of benzoylated zein in $D_2O/NaOD$.

Table 1
Acetylation of starch in ionic liquid

Biopolymer (g)	Ac_2O (g)/ Pyridine (g)	DS	Yield (%)	Reaction efficiency
1. Starch, 0.17	0.10/0.08	0.38	92	41
2. Starch, 0.17	0.20/0.16	0.76	73	41
3. Starch, 0.17	0.30/0.24	1.47	98	53
4. Starch, 0.17	0.41/0.32	1.88	100	50
5. Starch, 0.17	0.61/0.48	2.66	90	47

We also prepared the acidic IL, 1-methylimidazolium tetrafluoroborate (Zhu, Yang, Tang, & He, 2003) that promotes esterification without any catalyst. However, starch did not dissolve in it. Finally we prepared the BMIMdca (Forsyth, MacFarlane, Thomson, & von Itzstein, 2002; Liu, Janssen, van Rantwijk, & Sheldon, 2005) that is a liquid at room temperature and reportedly catalyze acylation of sugars without any base added. We found that starch was soluble in this solvent only at 90 °C. However, the acylation with acetic anhydride without pyridine did occur with low reaction efficiency and it gave acetylated starch with maximum DS of 0.3.

Low melting, deep eutectic mixtures were also evaluated as low cost alternatives to ionic liquids. Compositions and solubility characteristics of starch in these mixtures is shown in Table 2. Gelation of starch (and hence starch

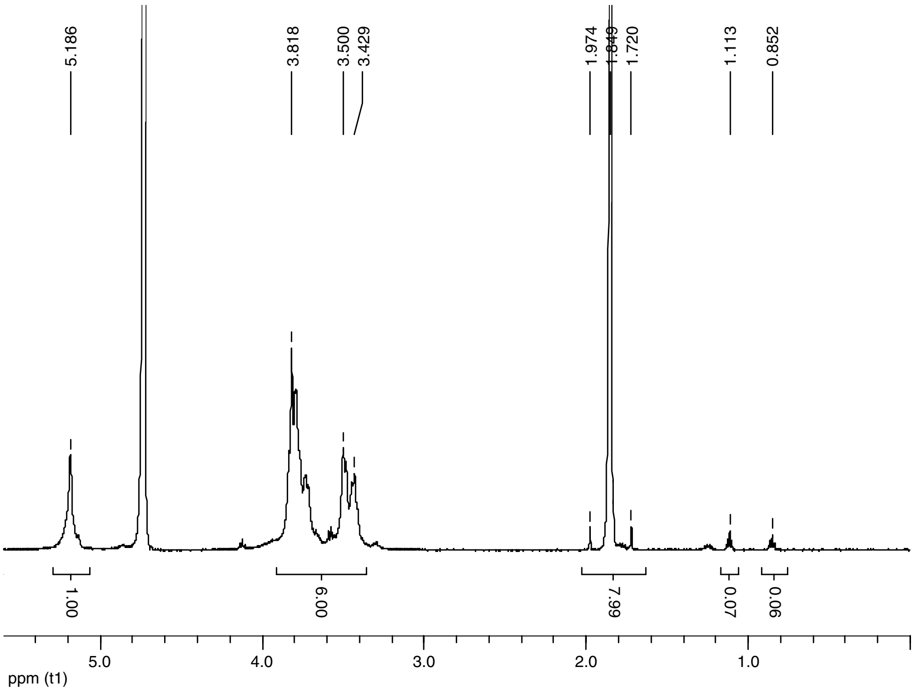

Fig. 4. Proton NMR of starch acetate (DS 2.6), entry # **1**, in D_2O/NaOD.

Table 2
Phase behavior of starch in deep eutectic solvent mixtures

Component A (g)	Component B (g)	Biopolymer (g)	Temperature (°C)	Appearance
Urea, 4.0	Calcium chloride, 1.0	Starch, 1.0	80	Gelled
Urea, 2.0	Choline chloride, 2.0	Starch, 0.4	100	Viscous
Choline chloride, 1.4	Citric acid, 1.9	Starch, 0.3	100	Viscous
Choline chloride, 1.4	Zinc chloride, 2.7	Starch, 0.2	98	Viscous
Choline chloride, 1.4	Oxalic acid, 0.9	Starch, 0.23	100	Brown solution

swelling and destructurization) were noted in mixtures of urea/$CaCl_2$, urea/choline chloride, choline chloride/$ZnCl_2$, and citric acid/choline chloride after heating to 80–100 °C. Less viscous, brown solutions were observed for starch in oxalic acid/choline chloride at 100 °C, suggesting some starch hydrolysis in this case. No starch solubilization (turbid suspensions) was noted for urea/NH_4Cl, succinic acid/choline chloride, maleic acid/choline chloride, phenylacetic acid/choline chloride mixtures. Zein had no apparent solubility in any of these solvent mixtures. Recently, cellulose was also reported to be solubilized by choline chloride/zinc chloride mixtures (Abbott, Bell, Handa, & Stoddart, 2005).

4. Conclusions

We have found that at 80 °C biopolymers, such as starch and zein, are soluble in ionic liquid 1-butyl-3-methylimidazolium chloride up to 15% (w/w) concentration. The starch solution was reacted with acetic anhydride and pyridine to give acetylated starch with DS of 0.3 to 2.6 in good yields. Similarly, zein solution was reacted with benzoyl anhydride and pyridine to give benzoate ester. In this work, we have demonstrated for the first time that IL could be used as a solvent for the chemical modifications of starch and zein. It was also found that starch dissolved in choline chloride/oxalic acid or choline chloride/$ZnCl_2$ in 5–10% concentration. However, zein was insoluble in choline chloride containing solvent systems.

Acknowledgements

We gratefully acknowledge Dr. Karl Vermillion for the NMR work, and Janet Berfield and Kelly Utt for carrying out the reactions.

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